510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY ONLY

I Background Information:

A 510(k) Number

K243499

B Applicant

NG Biotech

C Proprietary and Established Names

NG-Test CTX-M MULTI

D Regulatory Information

Product Code(s)	Classification	Regulation Section	Panel
PTJ	Class II	21 CFR 866.1640 - Antimicrobial Susceptibility Test Powder	MI - Microbiology

II Submission/Device Overview:

A Purpose for Submission:

To obtain a substantial equivalence determination for the qualitative detection of CTX-M enzymes from pure colonies of Enterobacterales.

B Measurand:

CTX-M enzymes (groups 1, 2, 8, 9, and 25)

C Type of Test:

Qualitative immunochromatographic assay (lateral flow)

III Intended Use/Indications for Use:

A Intended Use(s):
See Indications for Use below.

B Indication(s) for Use:
NG-Test CTX-M MULTI is an in vitro rapid and visual immunochromatographic assay for the qualitative detection of CTX-M enzymes (groups 1, 2, 8, 9, and 25) from pure colonies of Enterobacterales suspected of ESBL production when grown on the following media:

• 5% sheep blood agar or MacConkey agar (16-24 hours)

• HardyCHROM ESBL agar (18-24 hours)

The NG-Test CTX-M MULTI is intended as an aid for infection control in the detection of CTX-M enzymes-producing organisms (Enterobacterales) in healthcare settings. NG-Test CTX-M MULTI is not intended to guide or monitor treatment. A positive or negative NG-Test CTX-M MULTI test result does not rule out the presence of other mechanisms of antibiotic resistance. NG-Test CTX-M MULTI should be used in conjunction with other laboratory tests including phenotypic antimicrobial susceptibility testing.

C Special Conditions for Use Statement(s):

- Rx - For Prescription Use Only
- The performance of NG-Test CTX-M MULTI was established with colonies from blood agar, MacConkey agar, and HardyCHROM ESBL agar. Performance with other culture media has not been evaluated and is therefore unknown.
- The performance of NG-Test CTX-M MULTI with bacteria other than Enterobacterales has not been evaluated.
- Organism identification and elevated third-generation cephalosporin MICs should be determined prior to testing with NG-Test CTX-M MULTI.

D Special Instrument Requirements:

None (results are read manually/visually)

IV Device/System Characteristics:

A Device Description:

NG-Test CTX-M MULTI is an *in vitro* rapid and visual immunochromatographic assay for the qualitative detection of CTX-M enzymes (groups 1, 2, 8, 9, and 25) from pure colonies of Enterobacterales suspected of extended spectrum beta-lactamase (ESBL) production after culturing on agar and processing in an extraction buffer. The device consists of a sample port, sample and conjugate pad, and nitrocellulose test strip, which are contained within a plastic cassette, in addition to reagents for liquid extraction.

B Principle of Operation:

Monoclonal antibodies that recognize the five major CTX-M groups (groups 1, 2, 8, 9, and 25) are immobilized on a nitrocellulose membrane. Free monoclonal antibodies are present in the conjugate pad and labeled with colloidal gold. Colonies are mixed with extraction buffer to lyse

the bacteria then added to the sample pad. Upon addition of the processed sample to the sample pad, the capillary action of the nitrocellulose draws the sample through the mobile antibodies in the conjugate pad and the immobile antibodies on the test strip. The immobilized control antibodies capture any mobile antibodies that do not bind to the test line. The result can be read 15 minutes after adding the sample to the sample well. A positive result on the NG-Test CTX-M MULTI occurs when two red lines appear, one on the control (C) region and one on the test (T) region. A negative result occurs when only the control line is observed and indicates the sample does not contain any target CTX-M enzymes, or the CTX-M enzymes are present at a non-detectable level. If the control line does not appear, the test result is invalid.

V Substantial Equivalence Information:

A Predicate Device Name(s):
NG-Test CARBA 5

B Predicate 510(k) Number(s):
K191889

C Comparison with Predicate(s):

Device & Predicate Device(s):	Device: K243499	Predicate: K191889
Device Trade Name	NG-Test CTX-M MULTI	NG-Test CARBA 5
General Device Characteristic Similarities		
Intended Use/ Indications for Use	NG-Test CTX-M MULTI is an in vitro rapid and visual immunochromatographic assay for the qualitative detection of CTX-M enzymes (groups 1, 2, 8, 9, and 25) from pure colonies of Enterobacterales suspected of ESBL production when grown on the following media: • 5% sheep blood agar or MacConkey agar (16-24 hours). • HardyCHROM ESBL agar (18-24 hours). The NG-Test CTX-M MULTI is intended as an aid for infection control in the detection of CTX-M enzymes-producing organisms (Enterobacterales) in healthcare settings. NG-Test CTX-M MULTI is not intended to guide or monitor treatment. A positive or negative NG-Test	NG-Test CARBA 5 is an in vitro rapid and visual multiplex immunochromatographic assay for the qualitative detection and differentiation of five common carbapenemases (KPC, OXA-48-like, VIM, IMP, and NDM) from carbapenem non-susceptible pure bacterial colonies when grown on the following media: • 5% sheep blood agar or MacConkey agar (16-24 hours) for testing Enterobacterales (formerly *Enterobacteriaceae*) and *Pseudomonas aeruginosa*. • HardyCHROM CRE agar (18-24 hours) for testing *E. coli* and KES

	CTX-M MULTI test result does not rule out the presence of other mechanisms of antibiotic resistance. NG-Test CTX-M MULTI should be used in conjunction with other laboratory tests including phenotypic antimicrobial susceptibility testing.	(*Klebsiella aerogenes, Klebsiella oxytoca, Klebsiella pneumoniae, Enterobacter cloacae* complex and *Serratia marcescens*). The NG-Test CARBA 5 is intended as an aid for infection control in the detection of carbapenemase-producing Enterobacterales and *Pseudomonas aeruginosa* in healthcare settings. NG-Test CARBA 5 is not intended to guide or monitor treatment for carbapenem non-susceptible bacterial infections. A positive or negative NG-Test CARBA 5 test result does not rule out the presence of other mechanisms of antibiotic resistance. NG-Test CARBA 5 should be used in conjunction with other laboratory tests including phenotypic antimicrobial susceptibility testing.
Interpretation	Visual (manual)	Same
Controls	Built-in procedural control in every test strip	Same
Intended Culture Media	5% sheep blood agar, MacConkey agar	Same
Sample Type	Bacterial colonies	Same
Inoculum Preparation	By touching 3 colonies with a 1 µL loop	Same
General Device Characteristic Differences		
Intended Culture Media	HardyCHROM ESBL agar	HardyCHROM CRE agar
Analyte	CTX-M enzymes	Carbapenemase enzymes (KPC, OXA-48-like, VIM, IMP, NDM)

VI Standards/Guidance Documents Referenced:

- CLSI M100, 34th ed., "Performance Standards for Antimicrobial Susceptibility Testing".
- CLSI M02, 13th ed., "Performance Standards for Antimicrobial Disk Susceptibility Tests".

VII Performance Characteristics (if/when applicable):

A Analytical Performance:

1. Precision/Reproducibility:
The reproducibility of NG-Test CTX-M MULTI was evaluated in a study conducted with three lots of tests at three sites including one internal site. A panel of 20 well-characterized strains grown on 5% sheep blood agar (blood agar) and MacConkey agar was tested over five separate days. The panel included an equal mix of isolates that were determined prior to the study to be positive (i.e., harboring CTX-M) or negative (i.e., not harboring CTX-M) **(Table 1)**. The CTX-M status determination was based on molecular characterization and AST disk diffusion. Two independent readers, blinded to the panel contents and each other's results, conducted testing following the package insert and reviewed the test results from both types of agar media. A total of 300 positive results were expected (3 sites x 2 operators x 5 days x 10 strains that harbor CTX-M genes), and a total of 300 negative results were expected (3 sites x 2 operators x 5 days x 10 strains that do not harbor CTX-M genes).

Table 1: List of Strains Used in NG-Test CTX-M MULTI Reproducibility Study

Organism	Strain ID	Molecular Summary	CTX-M Group	Expected Results
Enterobacter cloacae	CDC 0501	CTX-M-9, TEM-1B, VIM-1	9	Positive
Enterobacter cloacae	IHMA 871550	CTX-M-9	9	Positive
Enterobacter cloacae	CDC 0502	IMP-8, SHV-12, TEM-1B	N/A	Negative
Enterobacter cloacae	IHMA 958153	ACT-32, IMP-8, SHV-12(2be), TEM-OSBL(2b)	N/A	Negative
Escherichia coli	IHMA 959898	CTX-M-15, CTX-M-40	1, 8	Positive
Escherichia coli	IHMA 958085	CTX-M-116	1	Positive
Escherichia coli	LSI 971	NDM-1, SHV-12, TEM-1	N/A	Negative
Escherichia coli	IHMA 951011	CMY-42, NDM-5, TEM-OSBL(2b)	N/A	Negative
Klebsiella oxytoca	IHMA 870581	CTX-M-30, CTX-M-75	1, 2	Positive
Klebsiella oxytoca	LSI 4744	CTX-M-14, DHA-1, OXA, tet(A)	9	Positive
Klebsiella oxytoca	CDC 0375	SHV-5	N/A	Negative
Klebsiella oxytoca	IHMA 949841	ACC-1, NDM-1, TEM-OSBL(2b)	N/A	Negative
Klebsiella pneumoniae	IHMA 984357	CTX-M-124, SHV-OSBL(2b)	2	Positive
Klebsiella pneumoniae	IHMA 869028	CTX-M-14, TEM-129(2be)	9	Positive
Klebsiella pneumoniae	IHMA 845661	KPC-3, SHV-11(2b), TEM-1(2b)	N/A	Negative
Klebsiella pneumoniae	IHMA 928329	KPC-3, SHV-12(2be), TEM-OSBL(2b)	N/A	Negative
Proteus mirabilis	IHMA 930043	CTX-M-32	1	Positive
Proteus mirabilis	IHMA 874812	CTX-M-79, TEM-1(2b)	1	Positive
Proteus mirabilis	IHMA 966438	DHA-1, IMP-26	N/A	Negative
Proteus mirabilis	IHMA 1104843	CMY-16, TEM-OSBL(u), VIM-1	N/A	Negative

Across all five days of the study, 100% of the isolates yielded results in agreement with the expected positive and negative outcomes for NG-Test CTX-M MULTI from both blood agar and MacConkey agar at all three sites (**Tables 2** and **3).** The reproducibility of NG-Test CTX-M MULTI was determined to be acceptable**.**

Table 2: Summary of Reproducibility Results from Blood Agar

Day	*N*	NG-Test CTX-M MULTI Positive	%*	*N*	NG-Test CTX-M MULTI Negative	%*
1	60	60	100.0	60	60	100.0
2	60	60	100.0	60	60	100.0
3	60	60	100.0	60	60	100.0
4	60	60	100.0	60	60	100.0
5	60	60	100.0	60	60	100.0
Overall	**300**	**300**	**100.0**	**300**	**300**	**100.0**

* % Calculated as percent of observed/expected

Table 3: Summary of Reproducibility Results from MacConkey Agar

Day	*N*	NG-Test CTX-M MULTI Positive	%*	*N*	NG-Test CTX-M MULTI Negative	%*
1	60	60	100.0	60	60	100.0
2	60	60	100.0	60	60	100.0
3	60	60	100.0	60	60	100.0
4	60	60	100.0	60	60	100.0
5	60	60	100.0	60	60	100.0
Overall	**300**	**300**	**100.0**	**300**	**300**	**100.0**

* % Calculated as percent of observed/expected

2. Linearity:
   Not applicable

3. Analytical Specificity/ Cross Reactivity:
   The analytical specificity of the NG-Test CTX-M MULTI was evaluated using organisms that possess antimicrobial resistance mechanisms other than CTX-M or are not susceptible to third-generation cephalosporins (cefotaxime, ceftazidime, ceftriaxone, cefpodoxime) and other agents (aztreonam and cefoxitin). Some of the antimicrobial resistance mechanisms included were TEM, SHV, VEB, ACT, CMY, DHA, FOX, MIR, ACC, VIM, KPC, IMP, NDM, and OXA. A panel of 55 organisms was tested after growth on blood agar and MacConkey agar (including 2 *Citrobacter freundii*, 3 *Enterobacter asburiae*, 7 *Enterobacter cloacae*, 13 *Escherichia coli*, 4 *Klebsiella oxytoca*, 15 *Klebsiella pneumoniae*, 1 *Morganella morganii*, 7 *Proteus mirabilis*, and 3 *Serratia marcescens*).

   For evaluation of colonies grown on HardyCHROM ESBL agar, a panel of 50 strains intended for use with this agar medium were seeded in raw stool or stool in C&S Cary Blair Transport Media, followed by sub-culture and testing (including 1 *Citrobacter freundii*, 3 *Enterobacter asburiae*, 7 *Enterobacter cloacae*, 12 *Escherichia coli*, 4 *Klebsiella oxytoca*, 14 *Klebsiella pneumoniae*, 1 *Morganella morganii*, 5 *Proteus mirabilis*, and 3 *Serratia marcescens*; 5 strains included for testing with blood and MacConkey were susceptible to the antimicrobial agents in the agar, thus were not evaluated from the HardyCHROM ESBL agar).

The organism groups and their relevant molecular characteristics used in the analytical cross-reactivity study is provided in **Table 4**. All non-target organisms evaluated from blood (55/55, 100%), MacConkey (55/55, 100%), and HardyCHROM ESBL (50/50, 100%) agar yielded a negative NG-Test CTX-M MULTI result. The cross-reactivity of the NG-Test CTX-M MULTI was determined to be acceptable.

Table 4: Resistant mechanisms evaluated for Cross-Reactivity

Organism	Resistance Mechanisms Evaluated	
	Blood & MacConkey Agar	**HardyCHROM ESBL Agar**
Citrobacter freundii	CMY-41, CMY-49, IMP-8, KPC-2, TEM-OSBL(2b)	CMY-41, IMP-8, TEM-OSBL(2b)
Enterobacter asburiae	ACT-2, IMP-14, MIR-8, NDM-1, OXA-48(c), TEM-OSBL(2b), VEB-2	ACT-2, IMP-14, MIR-8, NDM-1, OXA-48(c), TEM-OSBL(2b), VEB-2
Enterobacter cloacae	ACT-17, ACT-32, ACT-45, ACT-type, DHA-1, FOX-5, IMP-1, IMP-8, KPC-2, KPC-3, OXA-1, OXA-9, SHV-12, SHV-12(2be), TEM-1A, TEM-1B, TEM-OSBL(2b)	ACT-17, ACT-32, ACT-45, ACT-type, DHA-1, FOX-5, IMP-1, IMP-8, KPC-2, KPC-3, OXA-1, OXA-9, SHV-12, SHV-12(2be), TEM-1A, TEM-1B, TEM-OSBL(2b)
Escherichia coli	CMY-2, CMY-42, KPC-3, NDM-1, NDM-5, OXA-1, OXA-30, OXA-48(c), SHV-12, TEM-1, TEM-1(2b), TEM-6(2be), TEM-10, TEM-12(2be), TEM-20(2be), TEM-OSBL(2b)	CMY-2, CMY-42, KPC-3, NDM-1, NDM-5, OXA-1, OXA-30, SHV-12, TEM-1, TEM-6(2be), TEM-10, TEM-12(2be), TEM-20(2be), TEM-OSBL(2b)
Klebsiella oxytoca	ACC-1, NDM-1, SHV-5, SHV-7(2be), TEM-52(2be), TEM-OSBL(2b)	ACC-1, NDM-1, SHV-5, SHV-7(2be), TEM-52(2be), TEM-OSBL(2b)
Klebsiella pneumoniae	CMY-2, IMP-8, KPC-2, KPC-3, KPC-12, NDM-1, OXA-9, OXA-48(c), SHV-2A(2be), SHV-7, SHV-11, SHV-11(2b), SHV-12(2be), SHV-27(2b), SHV-55(2be), SHV-83(2b), SHV-90(2be), SHV-133(u), SHV-136(u), SHV-154, TEM-1, TEM-1(2b), TEM-4(2be), TEM-11(2be), TEM-141, TEM-OSBL(2b), VEB-1	CMY-2, IMP-8, KPC-2, KPC-3, KPC-12, NDM-1, OXA-9, SHV-2A(2be), SHV-7, SHV-11, SHV-11(2b), SHV-12(2be), SHV-27(2b), SHV-55(2be), SHV-83(2b), SHV-90(2be), SHV-133(u), SHV-136(u), SHV-154, TEM-1, TEM-1(2b), TEM-4(2be), TEM-11(2be), TEM-141, TEM-OSBL(2b), VEB-1
Morganella morganii	DHA-1	DHA-1
Proteus mirabilis	CMY-16, DHA-1, IMP-26, KPC-3, TEM-1A, TEM-15(2be), TEM-24(2be), TEM-92(2be), TEM-OSBL(u), VIM-1	CMY-16, DHA-1, IMP-26, TEM-15(2be), TEM-92(2be), TEM-OSBL(u), VIM-1
Serratia marcescens	CMY-16, FOX-5, IMP-47, OXA-2, OXA-10, TEM-1(2b)	CMY-16, FOX-5, IMP-47, OXA-2, OXA-10, TEM-1(2b)

4. Assay Reportable Range:
   Not applicable

5. Traceability, Stability, Expected Values (Controls, Calibrators, or Methods):

a) Agar Incubation Study

The ability of the NG-Test CTX-M MULTI to consistently provide correct results over the range of recommended agar incubation times was evaluated using five strains previously characterized to have a target CTX-M. The five strains were tested after growth on blood and MacConkey agar every two hours from 16 to 24 hours of incubation. The same five strains were also tested after growth on HardyCHROM ESBL agar every two hours from 18 to 24 hours of incubation. All organisms tested produced the expected result on NG-Test CTX-M MULTI at each time point tested.

b) Refrigerated Storage Study

To evaluate whether organism cultures (plated on agar media) stored in the refrigerator could be used with NG-Test CTX-M MULTI, strains were evaluated each day for three days after refrigeration. Five strains were tested after growth on blood agar, MacConkey agar, and HardyCHROM ESBL agar. All organisms tested produced the expected result on NG-Test CTX-M MULTI for each day of refrigeration.

c) Quality Control

Quality control testing was performed each day of the clinical study. The QC panel consisted of one positive control organism and one negative control organism (**Table 5**). The QC testing gave the expected results each day of testing.

Table 5: Organisms for NG-Test CTX-M MULTI QC Testing

QC Organism	Expected Test Line Result
Escherichia coli, NCTC 13353[1]	Positive
Escherichia coli, ATCC 25922	Negative

[1]QC organism shall be maintained on blood agar with a ceftriaxone (CRO) disk to ensure the phenotype does not change, i.e. no ESBL plasmid loss occurs.

6. Detection Limit:

Analytical Reactivity
The analytical reactivity (inclusivity) of NG-Test CTX-M MULTI was evaluated using a panel of 57 strains of Enterobacterales characterized to have at least one target CTX-M enzyme **(Table 6)**. Each organism was inoculated onto 5% sheep blood agar (blood agar) and MacConkey agar plates. Additionally, 56 of these strains were inoculated on HardyCHROM ESBL (HC ESBL) agar after being seeded in raw/unpreserved stool or stool preserved in C&S Cary Blair transport medium. Blood and MacConkey agar plates were incubated at 35°C for 16-24 hours, and HC ESBL agar plates were incubated for 18-24 hours. Each isolate was tested with the NG-Test CTX-M MULTI test in triplicate from each media type in line with the instructions for use.

The percent agreement for all target organisms evaluated was 100% (57/57), 100% (57/57) and 100% (56/56) from blood, MacConkey, and HC ESBL agar, respectively **(Table 6).**

Table 6: Summary of Analytical Reactivity Study

Organism	Number of Strains Tested on Blood/MacConkey	Number of Strains Tested on HardyCHROM ESBL	CTX-M Groups and Number of Variants Tested	CTX-M Variants Tested	NG-Test CTX-M MULTI Percentage Agreement Among All Tested Media
Citrobacter species	1	1	Group 1 (*n*=1)	CTX-M-15	1/1 (100 %)
Enterobacter cloacae	9	9	Group 1 (*n*=4)	CTX-M-15, -22, -30	9/9 (100 %)
			Group 9 (*n*=4)	CTX-M-9, -9-like	
			Group 25 (*n*=1)	CTX-M-100	
Escherichia coli	19	19	Group 1 (*n*=11)	CTX-M-1, -3, -15, -55, -79, -116	19/19 (100 %)
			Group 2 (*n*=1)	CTX-M -2	
			Group 8 (*n*=1)	CTX-M-40	
			Group 9 (*n*=7)	CTX-M-14, -24, -27	
			Group 25 (*n*=1)	CTX-M-100	
Klebsiella oxytoca	5	5	Group 1 (*n*=3)	CTX-M -15, -22, -30	5/5 (100 %)
			Group 2 (*n*=1)	CTX-M -75	
			Group 9 (*n*=1)	CTX-M-14	
			Group 25 (*n*=1)	CTX-M -100	
Klebsiella pneumoniae	14	14	Group 1 (*n*=6)	CTX-M-3, -12, -15, -22, -64	14/14 (100 %)
			Group 2 (*n*=2)	CTX-M-74, -124	
			Group 8 (*n*=1)	CTX-M-40	
			Group 9 (*n*=6)	CTX-M-14, -14b, -38	
			Group 25 (*n*=1)	CTX-M-25	
Klebsiella variicola	1	1	Group 25 (*n*=1)	CTX-M-152	1/1 (100 %)
Kluyvera ascorbata	1	1	Group 2 (*n*=1)	CTX-M-124	1/1 (100 %)
Proteus mirabilis	6	5[1]	Group 1 (*n*=5)	CTX-M-3, -15, -32, -79	6/6 on Blood and MC, 5/5 on HC ESBL (100 %)[1]
			Group 25 (*n*=1)	CTX-M-160	
Serratia marcescens	1	1	Group 1 (*n*=1)	CTX-M-15	1/1 (100 %)

[1] One *P. mirabilis* strain with a characterized CTX-M-3 variant was susceptible to an antimicrobial agent in the HC ESBL formula thus was not evaluated with NG-Test CTX-M MULTI from HC ESBL.

Organisms were further analyzed by the CLSI ESBL Disk Test for comparison. Per the CLSI M100 document, 34th edition, the eligible isolates included *K. pneumoniae, K. oxytoca, E. coli*, and *P. mirabilis* with the following antimicrobial susceptibility profile:

- For *K. pneumoniae, K. oxytoca*, and *E. coli*: Cefpodoxime ≤17mm; Ceftazidime ≤22mm; Aztreonam ≤27mm; Cefotaxime ≤27mm; Ceftriaxone ≤25mm.
- For *P. mirabilis*: Cefpodoxime ≤22mm; Ceftazidime ≤22mm; Cefotaxime ≤27mm.

A summary of results is provided in **Table 7.**

Table 7: CLSI ESBL Disk Test Results Summary

Organism	Number of Strains Tested	CTX-M Groups Tested	CTX-M Variants Tested	Positive CLSI ESBL Disk Test Results	Indeterminate CLSI ESBL Disk Test Results[1]	Number of Ineligible Isolates for the CLSI ESBL Disk Test[2]	Percentage of Isolates with Detected ESBL-Production via the CLSI ESBL Disk Test
Citrobacter species	1	1	CTX-M-15	N/A	N/A	1	N/A
Enterobacter cloacae	9	1, 9, 25	CTX-M-15, -22, -30, -9, -9-like, -100	N/A	N/A	9	N/A
Escherichia coli	19	1, 2, 8, 9, 25	CTX-M-1, -2, -3, -14, -15, -24, -27, -40, -55, -79, -100, -116	16	3	0	84%
Klebsiella oxytoca	5	1, 2, 9, 25	CTX-M-14, -15, -22, -30, -75, -100	5	0	0	100%
Klebsiella pneumoniae	14	1, 2, 8, 9, 25	CTX-M-3, -12, -14, -14b, -38, -40, -64, -74, -124, -15, -22, -25	13	1	0	93%
Klebsiella variicola	1	25	CTX-M-152	N/A	N/A	1	N/A
Kluyvera ascorbata	1	2	CTX-M-124	N/A	N/A	1	N/A
Proteus mirabilis	6	1, 25	CTX-M-3, -15, -32, -79, -160	6	0	0	100%
Serratia marcescens	1	1	CTX-M-15	N/A	N/A	1	N/A

[1]Indeterminate results refer to cases where an increase of ≥ 5 mm in the zone diameter for either antimicrobial agent tested in combination with clavulanate, compared to the zone diameter of the agent tested alone (CTX vs. CTX-CLA and CAZ vs. CAZ-CLA), was not observed.

[2]Per the CLSI M100, the Enterobacterales eligible for the CLSI ESBL Disk Test are limited to *K. pneumoniae, K. oxytoca, E. coli*, and *P. mirabilis* with the following antimicrobial susceptibility profile: For K. pneumoniae, K. oxytoca, and E. coli: Cefpodoxime ≤17mm; Ceftazidime ≤22mm; Aztreonam ≤27mm; Cefotaxime ≤27mm; Ceftriaxone ≤25mm. For P. mirabilis: Cefpodoxime ≤22mm; Ceftazidime ≤22mm; Cefotaxime ≤27mm

The analytical reactivity of the NG-Test CTX-M MULTI for detection of CTX-M enzymes in Enterobacterales was determined to be acceptable.

7. Assay Cut-Off:

   Not applicable

B Comparison Studies:

1. Method Comparison with Predicate Device:

   Not applicable

2. Matrix Comparison:

   Not applicable

C Clinical Studies:

1. Clinical Sensitivity:

   The performance of the NG-Test CTX-M MULTI was evaluated in a clinical study that was conducted at three U.S. sites using a total of 309 Enterobacterales isolates, including 226 prospectively collected isolates (recovered fresh or less than 6 months prior to testing) and 83 retrospectively collected isolates (stock, recovered more than 6 months prior to testing). The NG-Test CTX-M MULTI performance was evaluated using bacterial colonies from these isolates grown on blood and MacConkey agar.

   To be enrolled in the study and included in the analysis of performance, retrospective isolates must have been (i) identified to the species level and (ii) determined to be non-susceptible to one or more 3rd generation cephalosporins by an FDA-cleared methodology, or contain a previously determined mechanism of resistance (CTX-M, TEM, SHV, AmpC, etc.). The isolates were from various geographical origins, including from both the United States and outside the United States. All prospective isolates of Enterobacterales were enrolled regardless of susceptibility to 3rd generation cephalosporins; a minimum of 50 prospective isolates were enrolled per site.

   The NG-Test CTX-M MULTI results for each isolate were interpreted using the combined reference algorithm as depicted in **Table 8**. The combined reference test comprised an FDA-cleared PCR test for detecting CTX-M-encoding genes and AST determination of resistance to at least one of the tested antimicrobial agents. Ceftriaxone disks were routinely used to maintain selective pressure for isolated colonies of retrospective Enterobacterales isolates.

Table 8: Composite Reference Method Algorithm for Interpretation of NG-Test CTX-M MULTI Results

PCR Result	AST[1]	Combined Reference (PCR & AST) Result	NG-Test CTX-M MULTI Result	NG-Test CTX-M MULTI Interpretation
+	NS	Positive	+	**True Positive**
+	S	Negative	+	**False Positive**
-	NS	Negative	+	**False Positive**
-	S	Negative	+	**False Positive**
+	NS	Positive	-	**False Negative**
+	S	Negative	-	**True Negative** – Lack of gene expression
-	NS	Negative	-	**True Negative** – Other mechanism of resistance is present
-	**S**	**Negative**	**-**	**True Negative**

[1] NS = Not susceptible (intermediate or resistant results to one or more antimicrobial agents tested per CLSI M100 Ed 34 breakpoints); S = Susceptible to all antimicrobial agents tested per CLSI M100 Ed 34 breakpoints.

A total of 311 Enterobacterales isolates were initially enrolled. One organism was excluded because it was unable to be recovered for testing by the clinical site. Additionally, one organism that was initially enrolled was excluded from the analysis because it was identified as *E. faecalis* and not an Enterobacterales upon subculture. Thus, a total of 309 isolates were included in the analysis from blood and MacConkey agar. A total of 308 isolates showed concordant results between the NG-Test CTX-M MULTI and comparator PCR testing combined with susceptibility results (Composite Reference Method). One false-positive result was observed. The analysis for NG-Test CTX-M MULTI is summarized in **Table 9**.

Table 9: Comparison of NG-Test CTX-M MULTI (Blood and MacConkey Agar) to Composite Reference Method

		Composite Reference Method		
		Positive	Negative	Total
NG-Test CTX-M	Positive	151	1[1]	152
	Negative	0	157	157
	Total	151	158	309
Positive Percent Agreement (PPA)		151/151 = **100%** (95% CI: 97.5-100%)		
Negative Percent Agreement (NPA)		157/158 = **99.4%** (95% CI: 96.5-99.9%)		

[1] One isolate of *Citrobacter amalonaticus* was positive on NG-Test CTX-M MULTI, not susceptible to at least one antimicrobial tested, and negative for blaCTX-M by PCR.

The CTX-M variants detected during the NG-Test CTX-M MULTI clinical study are outlined in **Table 10**.

Table 10: Overall CTX-M Variants Detected by NG-Test CTX-M MULTI in Clinical Study (Blood Agar and MacConkey Agar)

CTX-M Group	Overall Variants Detected in US Clinical Trial
1	CTX-M-1, -3, 15, -55, -178, -232, -V236A[1]
2	CTX-M-2
8	CTX-M-8
9	CTX-M-14, -24, -27, -65

[1] The CTX-M variant in this isolate features a V263A substitution (valine to alanine at position 263).

The performance of NG-Test CTX-M MULTI was further evaluated at a single internal site using bacterial growth on HardyCHROM ESBL in compliance with the intended use of this medium. A subset of 193 isolates were selected based on the intended use of the medium. The isolates comprised of recent isolates from the prospective and retrospective isolates collected during the CTX-M MULTI clinical evaluation that were available for testing and fall under HardyCHROM ESBL organism claims (i.e., ceftazidime and cefpodoxime non-susceptible Enterobacterales).

Isolates were seeded in both raw/unpreserved stool and stool preserved in C&S Cary Blair transport medium and plated on HardyCHROM ESBL agar. Colonies were then tested with the NG-Test CTX-M MULTI. Among the tested Enterobacterales, 192 showed concordant results between the NG-Test CTX-M MULTI and the composite reference method. One false-positive result was identified, corresponding to the same discrepant isolate from the testing with blood agar and MacConkey agar. The analysis for NG-Test CTX-M MULTI is summarized in **Table 11**.

Table 11: Seeded Study. Comparison of NG-Test CTX-M MULTI (HC ESBL Agar – Raw and C&S Stool) to Composite Reference Method

		Composite Reference Method		
		Positive	Negative	Total
NG-Test CTX-M	Positive	150	1[1]	151
	Negative	0	42	42
	Total	150	43	193
Positive Percent Agreement (PPA)		150/150 = 100% (95% CI: 97.5-100%)		
Negative Percent Agreement (NPA)		42/43= 97.7% (95% CI: 87.9[2]-99.9%)		

[1] One isolate of *Citrobacter amalonaticus* was positive on NG-Test CTX-M MULTI, not susceptible to at least one antimicrobial tested, and negative for blaCTX-M by PCR.
[2] Lower bound is below 90% due to lower quantity of CTX-M negative clinical isolates that meet HardyCHROM ESBL agar claims. Additional CTX-M negative isolates were evaluated in the cross-reactivity study.

Table 12 shows the false-positive result of the NG-Test CTX-M MULTI compared to the reference method PCR combined with AST from all agar media evaluated. The clinical isolate of *Citrobacter amalonaticus* was resistant to cefotaxime, ceftriaxone, cefpodoxime, and aztreonam, according to the breakpoints described in the CLSI M100, 34th edition. The isolate was re-tested with NG-Test CTX-M MULTI and yielded positive results. A PCR re-test confirmed the negative PCR result.

Table 12: False-Positive Result of NG-Test CTX-M MULTI (Blood, MacConkey, and HC ESBL Agar) vs. Reference Method

Organism ID	**NG-Test CTX-M MULTI**	**PCR**	**AST (mm)[1]**						**Combined Reference (PCR & AST) Result**	**Interpretation**
			CTX	**CAZ**	**CRO**	**CPD**	**ATM**	**FOX**		
Citrobacter amalonaticus	Positive	Negative	21	27	16	15	13	26	Negative	False Positive

[1]CTX= cefotaxime; CAZ= ceftazidime; CRO= ceftriaxone; CPD= cefpodoxime; ATM= aztreonam; FOX= cefoxitin

The results were determined to be acceptable.

To address the indications for use, the following statements are included in the Limitations section of the device labeling:

The performance of NG-Test CTX-M MULTI was established with colonies from blood agar, MacConkey agar, and HardyCHROM ESBL agar. Performance with other culture media has not been evaluated and is therefore unknown.

The performance of NG-Test CTX-M MULTI with bacteria other than Enterobacterales has not been evaluated.

Organism identification and elevated third-generation cephalosporin MICs should be determined prior to testing with NG-Test CTX-M MULTI.

2. Clinical Specificity:
   Refer to Section VII C(1) above.

3. Other Clinical Supportive Data (When 1. and 2. Are Not Applicable):
   Not applicable

D Clinical Cut-Off:

Not applicable

E Expected Values/Reference Range:

Not applicable

VIII Proposed Labeling:

The labeling supports the finding of substantial equivalence for this device.

IX Conclusion:

The submitted information in this premarket notification is complete and supports a substantial equivalence decision.